# Intracellular Pteroylpolyglutamate Hydrolase from Human Jejunal Mucosa

ISOLATION AND CHARACTERIZATION\*

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Human jejunal intracellular pieroylpolyglutamate hydrolase was purified 30-fold from intestinal mucosa. The apparent molecular weight of the enzyme was 75,000 by Sephades G-200 rel filtration, and the isoelectric point was at pH 8.0. The enzyme was maximally active at pH 4.5 and was unstable at increasing temperatures, Intracellular pterovipolyglutamate bydroisse cleaved both terminal and internal 7-glutamate linkages. In contrast, brush-border piercylpolyglutamate hydrolase catalyzed the hydrolysis of only terminal y-glutamate linkages. The intracellular enzyme showed greatest affinity for the complete folic acid molecule with longer glutamate chains. Subcellulat fractionation studies showed the intracellular enzyme was localized in lysosomes. These data show that the properties of human jejunal intracellular ptercylpolyglutamate hydrolase are distinct from those of the brush-border enzyme but are similar to the properties of intracellular pteroylpolygiutamate bydrolase described in other tissues.

Pterovipolyglutamate hydrolases catalyze the hydrolysis of prerovipolygiutamates to derivatives with shorter clutamate chains. These enzymes have been described in the intestinal mucose of several different species. Only the intracellular form of purroylpolyglutamate hydrolase has been found in the intestinal mucosa of most animals, whereas two forms of the enzyme have been identified in human and pig intestinal mucose. The first is associated with the brush-border membrane, and the second is soluble and in the intracellular traction (1). We recently described the purification and properties of human brush-border prerovipolygiutemate hydrolase and showed that this enzyme is involved in the digestion of ptercylpolygiutamate, the predominant form of dietary folate (2). Relatively little is known, however, shout the properties of human intestinal intracellular ptercylpolyglutamate hydroisse and its possible role in folate metabolism. Our present objectives were to isolate and characterize intracellular ptercylpolyglutamete hydrolese from human intestinal mucosa and to compare its properties with those of the human intestinal brush-border enzyme. These data show distinct properties for each hydrolase and suggest that intracellular pteroylpolygonament hydrolese may play a role in cellular foliate metaliclism that is unrelated to the direction of dietary folates.

## EXPERIMENTAL PROCEDURES!

### RESULTS

Physical Imparise—intracellular parepholyphusmate photons was purified 30-fold (Table I). The apparent molecular weight was estimated by gel filtration to be 75,000. The incolerate point was at pH 8.0. Maximal activity of the enzyme occurred at pH 4.5 (Fig. 14) and at 65. °C (Fig. 18). The enzyme was unstable at 37 °C in pH 4.5 assay boffer aimed (Fig. 1C). However, the linearity of the product versus time curve to up to 4.6 min (Fig. 1D) indicated a protective effect of the obstruct at 37 °C and ensured the validity of the following the contraction of the obstruction of the contract of the obstruction of the contract of the obstruction of the obstruction of the contract of the obstruction of the contract of the obstruction o

Affinity for Substrate-The K. for PteGlus, determined from a Lineweaver-Burk plot, was 1.2 pm. Fig. 2 shows reciprocal plots of PteGlus bydrolysis in the presence of varied concentrations of PueGlus. This compound was a competitive inhibitor of the reaction, with a K; of 0.09 pM obtained from a replot of the 2 intercepts. PteGlu, also showed similar inhibition characteristics but had lower affinity for the ensyme, with a K; of 1.2 pM (data not shown). The effects of various PteGlu, moieties on the activity of the enzyme is shown in Table II. Complete inhibition of PteGlu, bydrolysis was observed with PteGlu, and PteGlu, at 0.1 mm, Both PteGlu and H.PteGlu at 0.1 mm caused a 15% inhibition and at 1.0 mm, a 60% inhibition. At 1 mm, purine and a-digletamets showed 50 and 30% inhibition, respectively. There was a slight inhibition by 1 mM p-aminohenzoylglutamic acid and no inhibition by 1 mm glutamic scid, o diglutamic scid, or a-triglutamic scid (Table II).

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<sup>&</sup>quot;Fortions of this paper including "Experimental Procedure" and Fig. 1-5) are piezaned in minipment at the rand of this paper. Mixipment is easily read with the sid of a transferd magnifying plane. Full uses photocopies are available from the Journal of Biological Commerty, 9600 foot-will Pile, Bethesda, MD 10614. Exquest Deument No. 660 Most Set (and the Moster, and include check on money and the first procedure of the procedure of the superior of the world Press of the commercial medicine of the footness that is a wealther from Warral Press.

<sup>&</sup>lt;sup>3</sup>The ebbrewhome used are PuGlia, purestirajouanie seidpuGlia, purestirprastivamie seid-PuGlia (bic seid-PuGlia, puroridiryusumie seid-HPuGlia, tetrabydropurcy)/louanie seid-Pu-Glia, purespolytitatima seid-PuGlia/PiGlia, puresyldirusumi/PiGjeuni-Pipudamie seid-PuGlia/PiGlialus, puresyldirusumi/PiGpieum-yldirusia-HPLC-hip pressure liquid-chrone-torpati-

TAPLE 1

Formal puritication of introvellular piercylipolygiatamate hydrolau						
From our	3 em.	Protein	Total	Specific activity	hecovery.	Punticetion tecsor
	PW.	my/m	miliante	multium de me	*	· /occ
10% homogenate"	4850	15.4	732.1	0.11	100	1
30,000 > g supernetant	1845	8.6	555	0.3	76	+ 1
pH precipitemen	940	4.1	480	0.51	65	3.4
(NH.), SO, precipitation	391	14.0	210	0.54	30-	3.€
leaders from a	24 *	1.4€	10K	4.14	14	27.€

"Homogenete was made from 20 g of tiesus

Table 11

Effect of different muleities of FueGlus, on PreGlus, hydrolysis

	5 of cortrol ectivity		
Cempound	0.1 may	1 1000	
Pu-Giu	85	36	
H.PteGlu	8!	40	
PtrGlu-	0	(	
PtrGlu-	C	(	
p-Aminobing oyightamic acid	90	8:	
Puerine	9€	41	
Glutamic ació	92	97	
7. Glutamylalowmic acic	100	70	
o-Glutamyisiutemic acid	100	100	
o-Glutemylelutemylelutemic acid	100	100	

Mechanism of Hydrobise—As shown in Fig. 3, the labeled products resulting from hydrolysis of PucGhil\*CyGlo were equal semounts of P\*C[plutamic acid, and \gamma\_r[butamy]!\*C[plutamic acid, rince incubations of the enzyme with PucGhilatonian acid, and the control of the control

Subscitutar Location—Using fresh issue, instructular puropipolyphismate hydriolas was localized in the fractions
enriched with mitochondria and lysosomes (Fig. SA). Fee ang
and thawing of the tissue resulted in a similar redistribution
of the lysosomal marker enzyme and intracellular purovpolyplutamate hydriolase (Fig. SB). More than 50% of both the
lysosomal marker N-acetylphicosominidase and pierovpolglutamate hydriolase separated in the soluble freetion. Other
marker enzymes showed no changer when compared to fresh
tissue.

#### DISCUSSION

The absorption of distary foliate is attributed in pair to the activity of specific pureopingly-pitamine byforioses located in the intestinal mucosa. To understand the mechanisms involved in abborption of distary foliate, we have focused our studies on two pureopingly-plusmate bydroleses in humaninestimal mucosa. Ricerathy, we reported on the purification and properties of the brush-border stream (2). In the pursual study, we have examined the properties study, we have examined the properties relationship of the two empires in foliate direction and metallicities of the two empires in foliate direction and me-

As shown in Table 1, a 50-fold posification of intucellolar perceyloply(iotamate hydrolass was exhibered. The rangem has an apparent molecular weight of 75,000, optimal excitors at pH 4,5, a pl of 5,0, and insubility as increasing temperature. The infinition of PucGlo, hydrolysis by PucGlo,  $(K_i = 1.5, M)$  and PucGlo,  $(K_i = 0.09, M)$  showed competitive infinition patterns with Linewever-though plots, indicative of pressure affinity for longer chain purelyloply(hutamates, Infinition of PucGlo, hydrolys) by PucGlo, and to a lesser strent by other

TABLE II;

Comparison of introvellular pie reylpolyplutamate hydroiase and arush-baraer pie mylpolyplutamate hydroiase.

Fropens	Introcellular	brush border	
Apparent M.	75,000	700,000	
H optimum.	4.5	6.5	
ol .	8.0	7.2	
furducing agent require i	Yes	No	
ment Temperature stability	Ne	Yes	
Metal requirement	No	Yee (Zn2", Co1")	
K. for PtrGlus (vM)	1.5	0.€	
K, for PtrGlus (µM)	0.05	9.0	
Mechanism of hydrolysis	Cleaves both terminal and internal linkages	Exopeptidam	
Final product	PteGlu	Pъ€Ghi	
Localination	Lyncacome	Frush border	

PteGlu- incubation support this conclusion. Subcellular frac-

tionation studies using differential centrifugation demon-

strated that the intracellular purroylpolyglutamate hydrolase

is located in the lysosomet. Comparisons of the properties of human intracellular and brush-border pterovipolyglutamete hydrolase indicate that they are distinct enzymes (Table III). The differences between these two enzymes include molecular weight, optimum pH, temperature stability, and requirement for metal ions and a reducing agent. Both enzymes showed similar K, values for PueGlus and greatest affinity when both the folic acid moiety and the y-givtamete bond were present. However, intracellular nurovipolygipus mate hydrolase had greater affinity for folstes with longer plutamete chains, whereas the brushborder enzyme had no preference for the number of glutamate residues. Whereas intracellular pterovipolyglutamate hydrolase is capable of cleaving both internal and terminal aglutamate linkages, the brush-border enzyme is an exopeptida₅€ (2).

Comparisons of human intestinal intracellular prevolpolcylutamate hydroises with previpolyphylutamate hydroises from other mammalian tissors reveal similarities and difference. Similar properties of precyplophylutamate hydroises have been described in human liver (16), bowne liver (17), rei liver (16), how kinder (18), pulsae primestine (20), and ramestine (21). In each site, the enzyme had an acidir pH optimum and was demonstrated to be lyconomia in human.

liver, not liver, and guines pig intestine. The ability to cleave internal a ciutamate bonds was observed in studies of pter cylpolyplutamete hydrolese reclated from bovine liver and ret intestine, whereas exopertiques activity was observed in hyman liver and nor kidney. Affinity toward longer plutamate chains was observed in both bovine liver and rat intestine Furthermore, sensitivity to sulfhydryl agents and the protective effect of reducing agents were properties of purpovipoly clutamete hydrolese from human liver, bovine liver, and hos kinney, which suggests involvement of SH groups in activity

The role of intracellular pterovipolygiutamate hydrolese in the human intestinal mucosa is obscure. A possible involve ment of the intracellular prerovipolyclutamete hydrogen it the absorption of dietary foliate is not excluded but would require transport of all or part of the pteroyipolypiutameter into the cell prior to hydrolysis. Intracellular purroylpolygiotemste hydrolase may function in regulating the levels of pteroylpolyglotameter within the enterocyte since others have demonstrated the canability for synthesis of these forms of the vitamin by intestinal mucosa (22). The similarities between human intestinal intracellular ptercylpolyglutamete hydrolase and the intracellular enzyme from other mammalian tissues imply that these enzymes have similar roles in cellular folase metabolism. Furthermore, preroylpolyplutamate is not only the preferred coenzyme for many foliatedependent enzymes in single carbon transfer reactions but also has been found to be an effective inhibitor of a number of enzymes, including thymidylate synthetase and methylene-H.PteGiu reductase (23, 24). Others observed increased riutamylation of folste in hepstoms cells in the presence of insulin or desamethasone (25). These observations surrest that a fairly complex regulation of ptercylpolyglutamete levels exists in the cell and implies that jejunal mucosal intracellular pterovipolycimemete hydrolese may play a significant physiological role in cellular metabolism.

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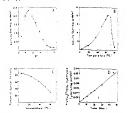
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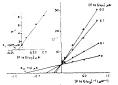
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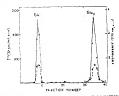


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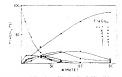
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